

Original Research Article

Investigating associative impact of Indoor Residual Spray and Insecticide Treated Nets for minimizing Visceral leishmaniasis vector population in Bihar (India).

ABSTRACT

Background- Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) are important tools in the fight against insect vectors of important diseases. In spite of having operational and logistic limitation(s), IRS and ITN are still in practice as conventional tools with mixed results for controlling *Phlebotomus argentipes*, the vector of Visceral Leishmaniasis (VL) in the Indian subcontinent. A combination of both tools might results better for reducing VL vector densities during the attack phase of the VL elimination initiative.

Methods- A comparison-based study was designed for analyzing the field efficacy of a combination strategy of IRS+ITN (PermaNet 3.0®) versus single interventions with IRS and ITNs alone comparing with control respectively. The study was conducted at the villages of Samastipur district of Bihar (India) using aspiration and light traps techniques for collecting sand fly. The numbers of all types of sand flies (male, unfed, fed and gravid female) were recorded during a period of 12 months after the intervention in the three study arms. The bioavailability of insecticide for IRS as well as ITN was also observed at the interval of 3 months with the help of cone bioassay technique established by World Health Organization (WHO).

Results- Lowest numbers and highest percent-reduction of sand flies (93.59% -100%) recorded from the sites with combined intervention of IRS+ITN as compared to the sites without any intervention at control (with 0% reduction) or with single control intervention either with IRS (4.29% - 86.77%) or with ITN (60.18% - 97.01%) followed by the reduction in bioavailability of insecticide in IRS and ITN over the period of time establishes the success of IRS plus ITN as combined approach for contaminating vector population. Also, the acceptance of the combined approach by the community was highest as compared to the single-intervention approaches.

Conclusions- Study results advocate for the use of the combined strategy during the attack phase of the VL elimination initiative in order to reduce or interrupt disease transmission. It may also be a valid approach during the maintenance phase in hot-spots of VL transmission.

Keywords: Visceral Leishmaniasis (VL) elimination, *Phlebotomus argentipes*, Indoor Residual Spray (IRS), Insecticide Treated Nets (ITNs), Integrated Vector Management (IVM).

1. INTRODUCTION

Visceral leishmaniasis (VL) is a notifiable protozoan parasitic disease [1] being transmitted by female sand fly *Phlebotomus argentipes* (Annandale and Brunetti; Diptera: Psychodidae) [2] and centralized within the marginalized community, mostly the rurals and tribals with low socio-economic status, with limited access to quality health care, communication, other basic amenities, lack of awareness on protection measures, and hence often referred as 'disease of the poor' and 'cause of poverty'[3]. For controlling VL and other vector borne disease, apart from the case detection and case treatment, Indoor Residual Spraying (IRS) with 5% Dichlorodiphenyltrichloroethane (DDT) at the concentration of 1gm/m² under vector control strategies remained mainstay for preventing this disease in India since 1953 [4]. Deficient operation of IRS and indiscriminate exploitation of DDT for vector control in endemic areas [5] along with logistic problems associated with sprayers and spray pumps [6-9] had contributed the reduced efficacy of IRS as well the decreased susceptibility of *P. argentipes* towards DDT [10-11] following the resurgence of sand flies just within 15 days of IRS [8].

Insecticide Treated Nets (ITNs) provide an alternate way for contaminating vector population [12]. Although insecticidal nets exhibited satisfactory results on impeding VL transmission as it only reduces the indoor vector population rather than the pathogenic female sand flies immigrating into the households [13] it may be more effective than the IRS [14-15]. In cluster-randomized controlled trials in India, Nepal and Bangladesh, these nets were found to control the VL vector population/houses by 24.9% (95% CI 1.80%–42.5%) [5,14-15]. These nets are treated with the pyrethroid insecticides at the manufacturing level and are largely resistant to multiple washes, facilitating its insecticidal longevity up to 3-5 years [16]. The number of limitations associated with single interventions of IRS or ITN hinders the expected results of targeting VL incidence through vector control. Combining the added benefits of ITN with IRS had remained untouched and underreported in case of VL vector. Therefore, taking inspiration from previous report of reduced vector population with the combo-intervention of IRS+ITN against malaria vectors [12] the study plan was designed accordingly for validating its effect in case of Indian VL vector.

2. MATERIAL AND METHODS

2.1. Study sites and study period.

The Samastipur district in Bihar state of India, covering an area of 2904 sq. kms with a total population of 425 million inhabitants is endemic for VL. Here the peaks for VL vector density exhibited during April to July and November to December [17]. On the basis of report obtained from the District Malaria Office (DMO) of Samastipur, four villages i.e., Mirzapur, Sahnitola, Nifsy were selected for IRS, ITN, IRS+ITN treatment respectively, and Bisanpur was allocated as Control site that remained untouched from any chemical treatment. Each village contained at least 100 households that were randomly selected. Therefore, overall 400 HHs were targeted for the study. The study period existed between October 2014 to October 2015 comprising baseline survey, sand fly density observation at study sites, 7 days prior intervention followed by the entomological data assessment, efficacy of intervention at regular interval during study session.

2.2. Baseline interview survey

At the beginning of the study, door-to-door surveys were conducted at the 4 villages by the trained interviewers using a questionnaire sheet. The questions were designed for obtaining information regarding the socio-economic status of the households and the protective measures taken against nuisance of insects.

2.3. Baseline survey on sand fly density

Sand fly collections were done using Aspiration Technique (AT) [18] as well as Light Trap Technique (LTT) [19] in order to get an optimal assessment over vectors density.

2.3.1. Aspiration Technique:

The diurnal resting sand flies were collected from cattle sheds, dark and damped corners of households during 0600 am to 0800 pm, by the team of expert insect collectors who had been imparted hands-on-training with the help of suction tubes and hand operated LED flashlights. The Borosil® glass test tubes were used for keeping captured sand flies. A cotton wool soaked in glucose solution was plugged on the mouth of these test tubes. These tubes were wrapped in semi-wetted cotton cloth and carefully transported to the laboratory.

Here, captured sand flies were released into the wooden-framed, wire meshed, cloth-lined Barraud cage (42×36×36 cm) for their adjustment to the new environment of insectary at Vector Biology and Control Division of this institute. After releasing captured sand flies, the *P. argentipes* were sorted out with the help of an identification key [20].

2.3.2. Light Trap Technique:

Nocturnal indoor sand flies were collected with a battery (John W. Hock Company, Model no. 300-6-220V) operated blower type miniature CDC light trap (John W. Hock Company, Model no. 512, developed by the Centers for Disease Control, U.S.). These CDC light traps were properly installed and adjusted during the day time, at the height of 15 cm away from the ground floor level and 3 cm away from the wall at the corner in the bedroom [21] or room where most of household members at each indexed

households used to sleep during the night, purposely for collecting sand flies. The battery was completely charged a day before the collection night, for its proper functioning during the period of 0600 pm to 0600 am hours under the supervision of field expert. Next morning, the light traps were de-installed and the dead insects collected in its collecting reservoir unit were transferred to the labeled Borosil® glass test tube, mouth tightly plugged with cotton ball. The insect samples were transported carefully to the laboratory where *P. argentipes* were identified and sorted out from the collections.

2.4. Insecticide Treated Nets (ITNs)

During the month of October 2014, in total 255 and 270 PermaNet® 3.0, treated with Deltamethrine (pyrethroid insecticide) at a dose of 2.8 g/kg $\pm$ 25% along with incorporation of piperonyl butoxide (25 g/kg $\pm$ 25%) as an insecticide synergist [22], were distributed in the villages for ITN (Sahnitola) and IRS+ITN (Nifsy) respectively so as to cover the entire sleeping spaces of the target populations. The average number of nets per house hold was 2.93 keeping the comfort of two adults and a child sleeping under a family size net. A village committee consisting of panchayat members (Governing council) and other opinion leaders were constituted to monitor proper use and maintenance of the nets.

2.5. Indoor Residual Spray (IRS)

Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna, Bihar, being affiliated by Indian Council of Medical Research, New Delhi, India supported the implementation of Indoor Residual Spray (IRS) activities with 4% DDT suspension in the districts of Bihar during October 2014, March 2015 and July 2015 using stirrup pumps following the guidelines under the national IRS policy [16].

2.6. Post intervention sand fly density evaluation

Adult sand flies were collected from the 6 randomly selected, indexed households (one room per house), from each cluster (comprising 100 households) targeted for interventions along with control. The same houses were deployed for collecting sand flies with AT as well as LTT throughout the study duration.

2.7. Feeding success rate in *P. argentipes*

Feeding success rate in *P. argentipes* was assessed by the visual observation of their feeding status [1, 2, 13] collected from the randomly selected 6 households from each cluster. Identification of male and female *P. argentipes* and distinction between fed and unfed female sand fly was done by scrupulous observation of abdominal appearance that served result for feeding success of the captured sand flies.

2.8. Assessing Bioavailability of Insecticide in Intervention (BII) by WHO cone method

The residual activity of IRS and ITN was measured at each interval of 3 months for estimating the Bioavailability of Insecticide in Intervention (BII) associated with IRS and ITN with the help of cone bioassay technique carried out in accordance with protocol led by WHO [23, 24] i.e., by exposing newly emerged *P. argentipes* (primarily female ones) directly to the (IRS & ITN) treated surfaces of 6 HHS selected from each cluster surface for 30 minutes and concluding susceptibility remarks by observing knockdown (after 30 minutes of exposure) and mortality (after 24 hours of exposure); and adjusting result by Abbot's formula [10, 25].

2.9. Assessing Percent Reduction due to Intervention (% RI) in vector density

The sand flies (of both sexes i.e., male and female), (fed, unfed, gravid among the female sand flies) were caught with the help of Aspiration Technique (AT) as well as through Light Trap Technique (LTT) from 4 study sites during the period of 13 months and on that basis of periodic collection of dead as well as living sand flies after intervention of IRS and ITN, % reduction due to intervention (%RI) was calculated for each months.

Percent Reduction due to Intervention (% RI) was assessed by measuring the magnitude of reduction in sand fly density at indexed intervened households and comparing those with pre-as well as post-treatment densities with the control houses in accordance with Mulla's formula [26].

2.10. Data entry and analysis

The data were analyzed using SPSS (IBM Statistics) Version 21 software and were transformed to obtain a normal distribution and homogeneity of variance. Significances of result were observed at 95% confidence intervals following the calculation of Mean Percent Reduction in sand flies due to Intervention (% RI) observed individually for each household from where *P. argentipes* had been collected. In addition Mann-Whitney-U test was applied for comparison between different interventions (IRS, ITN & IRS+ITN) to see the effect of interventions. P-Value <0.05 was considered statistically significant.

2.11. Post intervention survey for quantifying community acceptance, effectiveness and side effects perceived by the interventions.

Monthly house-to-house surveys were conducted for 12 months for ascertaining the proper usage of distributed nets. Also, physical status of nets at the study sites was observed during the monthly survey session. Using the net master list, all heads of households were interviewed. The questionnaires were used to determine people's perception of the benefits and/or side effects during use of ITNs and ITNs along with IRS. At the end of the study period after the 12th months of intervention, final discussion with the members of households in each intervention village was conducted for obtaining descriptive information on peoples' perception on the use of ITNs and IRS.

3. RESULTS

3.1. Socio-demographic characteristics of the study populations

The socio-demographic analysis before pre-intervention revealed that among the total population of 2878 at the targeted study sites, male population was slightly higher than the female population. Indicators of poverty were the following: Illiteracy rates were high and varied between 37% - 72%; among those with some kind of education, only a small proportion (9.43% - 15.78%) were graduated and above. The majority of houses in all study sites were thatched (38.5% - 55.1%) and mud plastered (9.2% - 21.7%) offering favorable living conditions to sand flies. Smaller proportions of households were of concrete (8.9% - 16.4%) as well as brick type (14.1% - 29.8%). The agricultural setting in all study areas displayed by the considerable proportion of houses with cattle sheds (26% - 57%) and the number of cattle owned by the household ranged from 66 - 153. Chemical measures taken by the local dwellers to protect themselves from nuisance insects (do-it-yourself IRS, self done IRS, bed nets, repellents) were present in roughly one quarter of houses; another quarter did not took any measures and almost half of the houses preferred both traditional and modern methods for avoiding the insects' nuisance as detailed in **Table 1**.

Table 1. Demographic and socio-economic conditions of the study populations and protective measures against nuisance of insects.

Village name & Id. No	Human Population				Education Profile			Cattle Assets		Measures for controlling insects' nuisance			
	Total population	Male population (%)	Female population (%)	Illiteracy (%)	Matric	Literacy (%) Intermediate	Graduate & Above	Total no. of cattle sheds	Total no. of cattles	Modern means (incl. Bednets, IRS, repellents)	Traditional means (incl. Smoking, oil spilling)	Both	None
Bisan Pur (Control)	814	55	45	58	57.24	33.33	9.43	26	98	22.14	15.73	5.73	56.4
Sahni Tola (ITN)	544	55	45	72	63.15	21.05	15.78	47	66	26.17	18.27	23.42	32.14
Mirza Pur (IRS)	857	51	49	37	68.25	26.98	4.76	57	153	24.16	29.44	14.35	32.05

Nifsy (IRS+ITN)	663	57	43	41	76.47	17.64	5.88	31	101	27.51	36.54	26	10.31
Total	2878	---	---	---	---	---	---	161	418	---	---	---	---

3.2. *P. argentipes* collection with AT and LTT

Of the total number of 679 *P. argentipes*, collected during 12 months from all sites, 33.43% (227) and 66.56% (452) were collected with the AT and LTT respectively. More sand flies were collected at night with the LTT in contrast to AT. During the night almost the same proportions of male and female sand flies were collected with LTT as unfed or gravid females are active at night in contrast to laziness of fed females that either rest on the wall or shelter in dark crevices for producing eggs via digestion of blood meal. During the day, particularly in early morning hours, more females were collected through the AT being most of them either gravid (42.75%) or fed with blood (44.22%) (Table 2).

Table 2. Classified sand fly collection during 12 months after insecticide intervention at the study villages.

	Aspiration Technique (N=227; 33.43%)				Light trap technique (N=452; 66.56%)			
	Male N=89; (39.20%)	Female N=138; (60.79%)			Male N=220; (48.67%)	Female N=232; (51.32%)		
		Fed N=61; (44.22%)	Unfed N=18; (13.04%)	Gravid N=59; (42.75%)		Fed N=16; (6.89%)	Unfed N=172; (74.13%)	Gravid N=44; (18.96%)
Bisan	40	27	4	32	88	8	88	16
Pur (Control)	(30.30%)	(44.26%)	(22.22%)	(54.23%)	(43.78%)	(50%)	(51.16%)	(36.36%)
Sahni	11	11	7	17	37	4	39	11
Tola (ITN)	(37.93%)	(18.03%)	(38.88%)	(28.81%)	(46.83%)	(25%)	(22.67%)	(25%)
Mirza	21	21	7	6	31	4	41	15
Pur (IRS)	(48.83%)	(34.42%)	(38.88%)	(10.68%)	(31.31%)	(25%)	(23.83%)	(34.09%)
Nifsy (IRS+ITN)	17	2	0	4	64	0	4	2
	(73.91%)	(3.27%)	(00.00%)	(6.77%)	(87.67%)	(00.00%)	(2.32%)	(4.54%)

Of the overall number of male sand flies, 39.20% were collected with the diurnal AT in contrast to 48.67% at night with LTT whereas, female sand flies' were more frequently collected (i.e., 60.79%) with diurnal AT as compared to the 51.32% recorded with nocturnal LTT. The highest collection of unfed female sand flies (74.13%) was recorded with the nocturnal LTT as compared to the diurnal AT (13.04%). In contrast, the highest percentage of fed and gravid female sand flies (44.22% and 42.75% respectively) were recorded with the AT as compared to those collected with LTT i.e., 6.89% and 18.96% respectively (Table 2). In present study, LTT effectively caught male and unfed female sand flies (i.e., 48.67% and 74.13% respectively) which seem to be active during the night in contrast to the AT capturing more blood-fed female sand flies (i.e., 44.22%) that, after feeding becomes inactive during the night and being caught during the early morning hours, after partial digestion of blood-meal. the findings were in accordance with the previous report [13]. Hence, it can be inferred that both techniques complement each other for trapping both types of sand flies depending upon their nature and it is beneficial to use them together.

3.3. Feeding success of *P. argentipes*

As the parasite transmission by *P. argentipes* depends upon the feeding success and hence reflects the chances of disease transmission. Therefore, female *P. argentipes* were collected during the post-intervention phase and their feeding status was verified and detailed in a tabular sheet (Table 2).

Out of all 679 sand flies collected from the intervention sites, 77 were fed female sand flies. Only 2 females were caught using AT from the village with the combined intervention (IRS and ITN) while the rest (75 fed female sand flies) were collected from the other villages including either of single interventions or control. Likewise only 6 gravid sand flies were collected in the combined intervention village and the other 97 gravid sand flies were collected from the villages with either ITN or IRS interventions (**Table 2**). In summary, After the intervention, the lowest numbers/proportions of sand flies were collected from villages with the combined approach (IRS plus ITN) as compared to single intervention sites (either IRS or ITN only) or the control site.

3.4. Bioavailability of the Insecticide in Intervention (BII) established by the WHO cone method

Quarterly evaluation of bioavailability of insecticide in intervention (BII) was accessed at intervals of 3 months after start of the intervention. Results were paralleled by the resurgence of the sand fly population after some months, probably due to reduction in the bioavailability of insecticidal content in both IRS as well as ITN. However, the reduction of insecticidal content of IRS was faster and more pronounced (corrected mortality ranged between 30.00-58.33) as compared to ITN (corrected mortality ranged between 77.78-91.25). The results are illustrated in **Table 3**.

Table 3. Result for Bioavailability of Insecticides in Intervention (BII) during the study period.

Months	No. of Sets	Indoor Residual Spray				Insecticide Treated Nets			
		No. of Sand fly Tested	% Test Mortality	% Control Mortality	Corrected Mortality	No. of Sand fly Tested	% Test Mortality	% Control Mortality	Corrected Mortality
(Bsaeline) October 2014	4	80	30.00	0.00	30.00	80	91.25	0.00	91.25
January 2015	3	45	55.56	6.67	52.38	45	84.44	0.00	84.44
April 2015	4	60	58.33	0.00	58.33	40	82.50	0.00	82.50
July 2015	3	36	50.00	8.33	45.45	36	77.78	0.00	77.78
October 2015	3	30	50.00	0.00	50.00	60	83.33	0.00	83.33

3.5. Percent Reduction (% RI) of sand fly density due to intervention.

The Monthly observation of percent reduction (% RI) of sand fly density due to intervention (**Table 4**) established the majority of reductions at a high significance ($p < 0.01$) that were observed in the IRS+ITN intervention arm as compared to the single intervention arms (IRS and ITN alone). However, due to the reduction in the bioavailability of insecticide, sand flies re-emerged after the initial reduction in sand fly density at the sites with either IRS or ITN alone, (**Table 4 and Figure 1**).

Table 4. Percent Reduction of sand flies due to Intervention with different intervention types in single and combined form.

Name of the study villages	MIRZAPUR		SAHANI TOLA		NIFY		P-Value Mann Whitney test (U-test) *c vs a c vs b
Type of intervention	IRS (a)		ITN (b)		IRS+ITN (c)		
	Mean (95% CI)	% RI	Mean (95% CI)	% RI	Mean (95% CI)	% RI	
IRS and ITN implementation on 12th- 14th October 2014							
Oct 2014	81.75 (70.6-92.8)	83.41	97.18 (93.4-100.9)	95.07	100 (100)	100	p<0.01 p<0.01
Nov 2014	82.40 (72.7-92.0)	83.34	97.00 (91.1-102.8)	97.07	98.9 (96.8-101.0)	98.44	p<0.01 p<0.05
Dec 2014	9.02 (-51.9-69.9)	26.06	87.71 (68.5-106.9)	87.73	96.7 (90.4-103.1)	93.59	p<0.01 p<0.05
Jan 2015	-65.33 (-279.6-148.9)	4.29	79.56 (59.5-99.5)	62.98	100 (100)	100	p<0.01 p<0.01
Feb 2015	14.97 (-20.0-50.0)	26.65	52.93 (5.08-100.7)	60.18	100 (100)	100	p<0.01 p<0.01
Mar 2015	82.65 (68.4-96.8)	86.77	89.68 (80.4-98.9)	87.48	97.84 (93.6-102.07)	98.14	p<0.05 p<0.05
Apr 2015	82.54 (70.2-94.8)	79.89	91.45 (79.9-102.9)	91.15	100 (100)	100	p<0.01 p<0.01
May 2015	83.88 (69.2-98.4)	86.53	85.58 (71.2-99.8)	85.38	100 (100)	100	p<0.01 p<0.01
Jun 2015	66.12 (49.2-82.9)	68.68	88.93 (79.05-98.8)	87.73	100 (100)	100	p<0.01 p<0.01
Jul 2015	62.66 (41.7-83.5)	68.40	87.22 (73.4-100.9)	88.67	100 (100)	100	p<0.01 p<0.01
Aug 2015	65.75 (39.8-91.6)	68.68	81.37 (60.6-102.0)	84.57	100 (100)	100	p<0.01 p<0.01
Sep 2015	73.55 (62.1-84.9)	73.77	82.65 (64.7-100.5)	85.76	100 (100)	100	p<0.01 p<0.01
Oct 2015	58.02 (12.2-103.8)	67.17	80.18 (53.5-106.8)	86.59	100 (100)	100	p<0.01 p<0.01

At the site with the combined treatment of IRS+ITN, no re-emergence of sand flies was recorded till 13 months following the intervention (**Figure 1**) however, overall % RI for IRS+ITN ranged between 93.59% - 100% during the study period (**Table 4** and **Figure 2**).

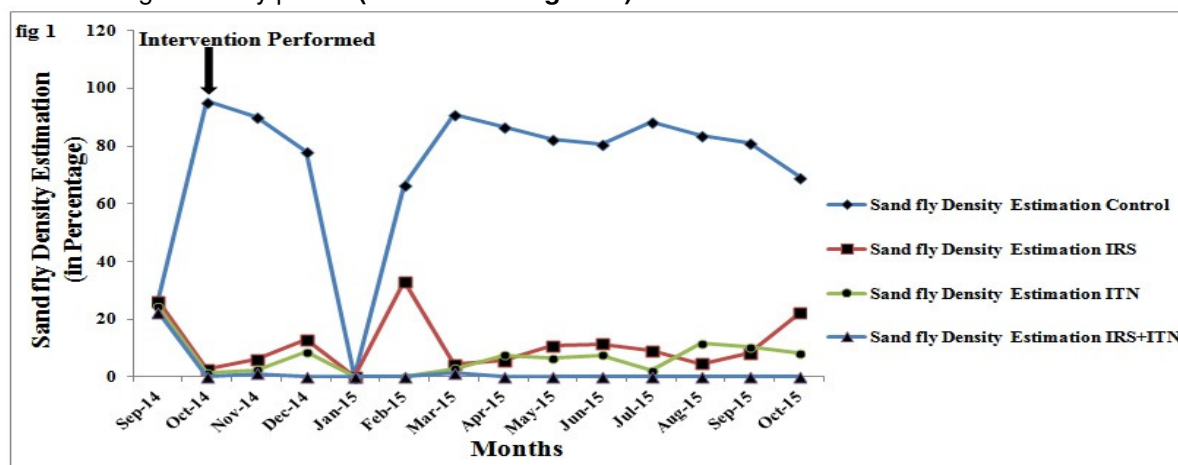


Figure 1. Graphical representation of sand fly density during Pre- and Post-intervention phase in the study villages.

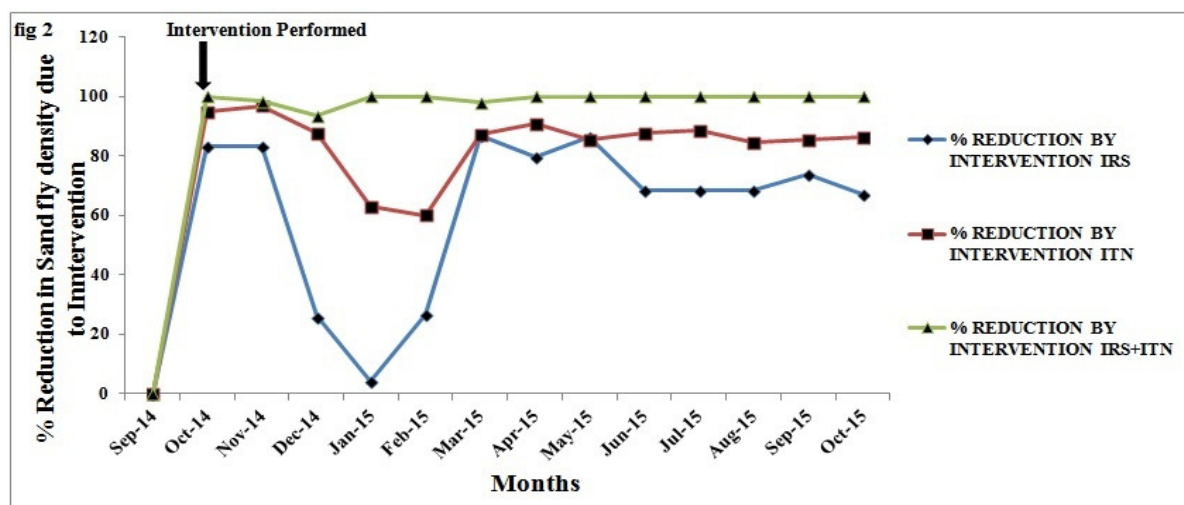


Figure 2. Comparative assessment of Percent Reduction (% RI) attributed due to intervention of IRS, ITN and IRS + ITN among *P. argentipes* during the period of 12 months, post intervention phase at the villages of Samastipur district, Bihar, India.

3.6. Perceived effectiveness and side effects by the community

The door-to-door survey was conducted among heads of the 400 targeted households along with additional 535 neighboring houses of intervened houses at the 4 study arms were interviewed for assessing the collateral effectiveness of interventions. All respondents facilitated with ITNs, confirmed the proper usage of provided bed nets as well as the continuous good physical conditions of the nets. This was confirmed by the village committee in-charge of supervising bed net use. Almost the only reported side effect was unpleasant smell, particularly in the 2 arms which included DDT spraying i.e., 83% and 91% in IRS and IRS+ITN respectively. Much less frequent was sneezing (10%, 6% and 12% in villages with either IRS or ITN or both respectively). Skin irritations were reported only in the villages which involved DDT spraying i.e., 8% and 11% in IRS and IRS+ITN respectively. The perception of added benefits (mainly reduction in nuisance of insects) was highest in villages where ITNs were involved (i.e., 97% against sand fly and mosquito bite and only in these sites, the disappearance of insects of other class viz., head lice, ants, cockroaches and house flies were also reported. Overall satisfaction was achieved in the villages involving ITNs as compared to the village with IRS as single intervention with 87% acceptability as illustrated in **Table 5**.

Table 5. Household survey after 12 months of Intervention for assessing peoples' perceptions of the interventions according to type of intervention received.

Serial No.	Questions	Proportion of Users (%)		
		IRS (N=857)	ITN (N=544)	IRS+ITN (N=663)
1	Do you sleep inside the provided nets?	NA	100	100
2	Do you felt any abnormality or suffocation while sleeping under nets?	NA	0	2
3	Did you suffer any of the following during the study period?			
a	Unpleasant Smell	83	76	91
b	Dizziness	0	2	0
c	Running nose	1	0	2
d	Fever	0	0	1
e	Headache	0	5	0
f	Sore Eyes	0	0	0.03

g	Skin Irritation	8	0	11
h	Coughing	0	0	0
i	Vomiting	0	0	2
j	Sneezing	10	6	12
k	Sleeplessness	0	0	0
4	Perceptions of additive benefits of Interventions			
a	Reduction in mosquito/ sand fly bites	39	83	97
b	Reduction in nuisance due to head lice.	0	80	83
c	Reduction in nuisance due to ants, cockroaches and house flies.	0	21	71
5	Will you continue sleeping under provided nets?	NA	100	100
6	Are you satisfied with the intervention?	87	100	100

4. DISCUSSIONS

Although substantial progress has been made regarding the VL elimination initiative by Bangladesh, Nepal and India [27] and particularly in India, financial and human resources have to be mobilized for the final push in order to interrupt VL transmission in villages with continued elevated VL incidence i.e., “hot-spots” and respond fast and effectively towards newly emerging foci. Though, several studies had exemplified the potency of IRS for reducing the vector population under controlled conditions [4] national IRS campaigns now had become a challenge [9] due various factors viz.,

- Uneven gap period between the two rounds of spray likely to hamper the residual efficacy of IRS [28].
- Community related factors like, acceptance and poor participation, complaints regarding the smell, irritation, etc., leaving behind white patches, toxicity for cats and other domestic animals [6, 8, 28].
- Programme related factors like, operational cuts in financial expenditure, irregular payment of spraying squads, lack of trained manpower, managerial problems, mismanagement and black-marketing of insecticide stock for agriculture purpose, lead to de-motivation and low quality performance on large –scale [28].
- Sub-optimal pumps and missing spare pieces [9].
- Side-effects of spray programme, perceived by the health of humans as well as other living-beings [28].

An alternative vector control measure is the use of insecticidal bed-nets, as VL vectors are night-biters (just as the African malaria vectors). Advantages of ITNs are that they are less costlier than IRS [29-30], they are well accepted by the community [15]; and can easily be distributed in PHCs settings or existing bed nets can be impregnated with slow release insecticides [15, 31].

Disadvantages of ITNs are, among others:

- They are less effective against VL vectors when compared to IRS in efficacy trials [4, 32] and did not have a significant impact on VL transmission in one study [14, 17].
- During hot summer months when local dwellers, particularly men, sleep outside their houses, special arrangements have to be made for fixing the nets.

Our study used, for financial reasons, a less powerful design than our preceding Cluster Randomized Trials (CRT) [5]. However, our comparison based study confirm that IRS, when applied by field staff of the vector control programme, effectively reduces VL vectors for a couple of months but then the effect is vanishing and after the end of 12th month, the vector population reached the same levels as before, corroborating the previous reports where the resurgence of sand flies was documented just after the 15 days of IRS [8]. Likewise ITNs reduced the vector populations for several months but then the effect slowly disappeared. Conversely, the combined approach (i.e., IRS plus ITNs) exhibited dramatic and long lasting effect for controlling the vector population [12, 33] and hence supports our study results.

5. CONCLUSIONS

Therefore in nutshell, The limitations with IRS and ITNs as single interventions can be overcome by a combined approach that should be rigorously collected through randomized controlled trials. This combined approach is particularly important during the attack phase of the elimination initiative in order to reduce or interrupt the disease transmission in VL endemic villages as well as new emerging foci (i.e. villages with new VL cases which were previously VL free). It may also be a valid approach during the maintenance phase in hot-spots of VL transmission.

9. CONSENT (WHERE EVER APPLICABLE)

Not required for this study

10. ETHICAL APPROVAL

This work had been performed under the project entitling 'Integrated vector management for the VL control vis-a-vis case study – A pilot study' approved by the Scientific Advisory Committee as well as Institutional Ethical Committee of Rajendra Memorial Research Institute of Medical Sciences (ICMR), Government of India in year 2013 with study reference no. INT 98 VBC 2013.

11. REFERENCES

1. Short HE, Barraud PJ, Crighead AC. Transmission experiments in Indian kala-azar with *Phlebotomus argentipes*. **Indian J Med Res.** 1927; 14:589-600.
2. Swaminath CS, Shortt HE, Anderson LAP. Transmission of Indian kala-azar to man by the bite of *P. argentipes* Annadale and Brunetti. **Indian J Med Res.** 1942; 30:473-477.
3. Redhu NS, Dey A, Balooni V, et al. Leishmania-HIV coinfection, an emerging problem in India. **AIDS.** 2006; 20:1213-1215.
4. Joshi A, Narain JP, Prasittisuk C et al. Can Visceral leishmaniasis be eliminated from Asia? **J Vector Borne Dis.** 2008; 45:105-111.
5. Joshi AB, Das ML, Akhter S et al. Chemical and environmental vector control as a contribution to the elimination of Visceral leishmaniasis on the India subcontinent: cluster randomized controlled trials in Bangladesh, India and Nepal. **BMC Med.** 2009; 5:7-54.
6. Kumar V, Kesari S, Kumar AJ et al. Vector density and the control of kala-azar in Bihar, India. **Mem Inst Oswaldo Cruz** 2009; 104:1019-1022.
7. Kumar V, Kesari S, Dinesh DS et al. A report on the indoor residual spraying (IRS) in the control of *Phlebotomus argentipes*, the vector of Visceral leishmaniasis in Bihar (India): an initiative towards total elimination targeting 2015 (Series-1). **J Vector Borne Dis** 2009; 46:225-229.
8. Chowdhary R, Huda MM, Kumar V et al. The Indian and Nepalese programmes of indoor residual spraying for the elimination of Visceral leishmaniasis: performance and effectiveness. **Ann Trop Med Parasitol.** 2011;105:31-35.
9. Huda MM, Mondal D, Kumar V et al. Toolkit for monitoring and evaluation of indoor residual spraying for Visceral leishmaniasis control in the India subcontinent: application and results. **J Trop Med.** 2011; 876742.
10. Rama A, Kumar V, Kesari S et al. Monitoring Susceptibility Status of *Phlebotomus argentipes* (Diptera: Psychodidae) at Bihar (India) for the Procurement of Homozygous DDT Resistant Colony. **J Trop Dis.** 2015; 3(4):170.
11. Coleman M, Foster GM, Deb R et al. DDT-based indoor residual spraying suboptimal for Visceral leishmaniasis elimination in India. **Proc Natl Acad Sci.** 2015; 112:8573-8578.
12. Kleinschmidt I, Schwabe C, Shiva M et al. Combining indoor residual spraying and insecticide-treated net interventions. **Am J Trop Med Hyg.** 2009; 81:519–524.
13. Dinesh DS, Das P, Picado A et al. Long-lasting insecticidal nets fail at household level to reduce abundance of sand fly vector *Phlebotomus argentipes* in treated houses in Bihar (India). **Trop Med Int Health.** 2008; 13:953–958.

14. Picado A, Das ML, Kumar V et al. Effect of Village-wide Use of Long-Lasting Insecticidal Nets on Visceral leishmaniasis Vectors in India and Nepal: A Cluster Randomized Trial. **PLoS Negl Trop Dis.** 2010; 4:e587.
15. Mondal D, Chowdhury R, Huda MM et al. Insecticide-treated bed nets in rural Bangladesh: their potential role in the Visceral leishmaniasis elimination programme. **Trop Med Int Health.** 2010; 15:1382-1389.
16. NVBDCP. Guidelines for ITNs and LLINs. <http://nvbdcp.gov.in/Doc/Guidelines-for-ITNS-LLINS.pdf>
17. Picado A, Das ML, Kumar V et al. *Phlebotomous argentipes* seasonal patterns in India and Nepal. **J Med Entomol.** 2010; 47:283-286.
18. Kumar V, Kesari SK, Sinha NK et al. Field trail of an ecological approach for the control of *Phlebotomus argentipes* using mud & lime plaster. **Indian J Med Res.** 1995; 101: 154-156.
19. Dinesh DS, Das P, Picado A et al. The efficacy of indoor CDC light traps for collecting the sandfly *Phlebotomus argentipes*, vector of *Leishmania donovani*. **Med Vet Entomol.** 2008; 22:120-123.
20. Lewis DJ. The phlebotomine sand flies (Diptera: Psychodidae) of the Oriental Region. **Bull Br Mus Nat Hist** 37. 1978.
21. Clements MF, Gidwani K, Kumar R et al. Measurement of Recent Exposure to *Phlebotomus argentipes*, the Vector of Indian Visceral Leishmaniasis, by Using Human Antibody Responses to Sand Fly Saliva. **Am J Trop Med Hyg.** 2010; 82(5), 801–807.
22. Technical brochure of Permanet 3.0. Technical basis for deployment of PermaNet® 3.0 in areas with pyrethroid-resistant malaria vectors. 2015. http://www.vestergaard.com/images/pdf/PN3_Tech_Eng_2015.pdf.
23. World Health Organization. Regional Strategic Framework for Elimination of kala-azar from the South-east Asia Region (2005-2015). SEA-VBC-85. 2005.
24. Mondal D, Chowdhury R, Huda MM et al. Insecticide-treated bed nets in rural Bangladesh: their potential role in the visceral leishmaniasis elimination programme. **Trop Med Int Health.** 2010; 15: 1382–1389.
25. Abbott WS. A method of computing the effectiveness of an insecticide. 1925. **J Am Mosq Control Assoc.** 1987; 3:302-3.
26. Mulla MS, Su T, Thavara U et al. Efficacy of new formulations of the microbial larvicide *Bacillus sphaericus* against polluted water mosquito in Thailand. **J Vector Ecol.** 1999; 24: 99-10.
27. World Health Organization. Elimination of Kala-azar: Report of the Fourth Meeting of the Regional Technical Advisory Group (RTAG) Kathmandu, Nepal. Document No. SEA-CD-243. Geneva: World Health Organization; 2012. https://apps.searo.who.int/pds_docs/B4811.pdf?ua=1
28. Sharma U, Singh S. Insect vectors of *Leishmania*: distribution, physiology and their control. **J Vector Borne Dis** 2008; 45:255–272.
29. Brännström AMP, Wolff C, Brännström N et al. Cost and cost effectiveness of long-lasting insecticide-treated bed nets - a model-based analysis. **Cost Eff Resour Alloc.** 2012; 10:5.
30. Uneze E, Nwadike G. Cost Effectiveness Analysis of Indoor Residual Spraying and Long Lasting Insecticidal Nets. 2013. http://gdn.int/admin/uploads/editor/files/GDN_PEMRP_CSEA_CEA_Health.pdf.
31. Mondal D, Huda MM, Karmoker MK et al. Reducing Visceral leishmaniasis by Insecticide Impregnation of Bed-Nets, Bangladesh. **Emerg Infect Dis.** 2013; 19:1131-34.
32. Dinesh DS, Das ML, Picado A et al. Insecticide Susceptibility of *Phlebotomus argentipes* in Visceral leishmaniasis Endemic Districts in India and Nepal. **PLoS Negl Trop Dis.** 2010; 4 :e859.
33. Kroeger A, Gonzalez JO, Avina AI. Malaria control reinvented: health sector reform and strategy development in Colombia. **Trop Med Int Health.** 2002; 7:450-8.

12. LIST OF ABBREVIATIONS

VL-	Visceral Leishmaniasis
IRS-	Indoor Residual Spraying
DDT-	Dichlorodiphenyltrichloroethane
ITN-	Insecticide Treated Nets
PHC-	Primary Health Care Centers

423 BSHS- Bihar State Health Society
424 DMO- District Malaria Office
425 CDC- Centre for Disease Control
426 EVM- Environmental Management
427 IGR- Insect Growth Regulator
428 DWL- Durable Wall Lining
429 LLIN- Long Lasting Insecticidal Nets
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